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Research paper

Bisbenzimidazole derivatives as potent inhibitors of the trypsin-like sites of the immunoproteasome core particle

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ABSTRACT

In this study, a monomeric (**MB**) and a dimeric (**DB**) bisbenzimidazoles were identified as novel proteasome inhibitors of the trypsin-like activity located on β2c sites of the constitutive 20S proteasome (IC_{50} values at 2–4 μM range). Remarkably, they were further shown to be 100- and 200-fold more potent inhibitors of the immunoproteasome trypsin-like activity (β2i sites, IC_{50} = 24 nM) than of the homologous constitutive activity. Molecular models of inhibitor/enzyme complexes in the two types of trypsin-like sites and corresponding computed binding energy values corroborated kinetic data. Different binding modes were suggested for **MB** and **DB** to the β2c and β2i trypsic sites. Each pointed to better contacts of the ligand inside the β2i active site than for β2c site. **MB** and **DB** represent the first selective inhibitors of the immunoproteasome trypsin-like activity described to date and can be considered as prototypes for inhibiting this activity.

1. Introduction

Constitutive proteasome (c26S) represents one of the most studied targets for anticancer drugs [1–7] together with cell cycle specific kinases, DNA polymerases, ribonucleotide reductases (RNR) and DNA-specific methyltransferases (DNA MTases) involved in hypermethylated states of tumor suppressor genes. The proteolytic function of c26S is confined to a barrel-shaped core particle (CP or c20S) bearing three duplicated catalytic sites: a caspase-like (C-L or PA), a trypsin-like (T-L) and a chymotrypsin-like (ChT-L). They lie respectively on the β1c, β2c and β5c subunits of the two stacked inner β rings, facing the catalytic chamber of the core particle [8–12]. Intensive work has been conducted to find inhibitors of this central catalytic core (c20S), searching for effects on the separate catalytic activities. A series of drugs with improved inhibitory activity on proteasome has been developed leading to successful clinical evaluations of bortezomib (PS-341 or Velcade^R) (Fig. 1A) for the treatment of multiple myeloma [13] and mantle cell lymphoma [14]. However, due to severe side effects of bortezomib treatments, probably associated with a high reactivity of this compound, a second generation of c20S proteasome inhibitors was developed. Carfilzomib (PR-171 or Kyprolis^R) [15] (Fig. 1A), has been more recently approved by FDA (2012). Analogs of the peptide boronate bortezomib (CEP-18770 [16] and MLN2238 [17]) and an analog of the epoxyketone carfilzomib (ONX912) [18–20] are under clinical evaluation according to their high potency on the c26S. A β-lactone, marizomib^R (salinosporamide A or NPI-0052) [21,22] (Fig. 1A) is also under clinical assays. All are highly potent inhibitors of the cChT-L activity (β5 subunit) and their action leads to covalent adducts on the catalytic Thr^1O^γ. Such a mechanism is also

Abbreviations: c20S, 20S constitutive proteasome catalytic core; cChT-L (β5c subunit), chymotrypsin-like; cT-L (β2c subunit), trypsin-like; cPA (β1c subunit), post-acid or caspase-like; i20S, 20S immunoproteasome catalytic core; iChT-L (β5i subunit), chymotrypsin-like; iT-L (β2i subunit), trypsin-like; iPA (β1i subunit), post-acid or caspase-like; AMC, 7-amino-4-methylcoumarin; β-NA, β-naphtylamide; DMSO, dimethyl sulfoxide; DMEM, Dulbecco Modified Eagle's Medium; IC_{50}, concentration giving 50 percent inhibition; PDB, protein data bank; CPK, Corey Pauling Koltun.

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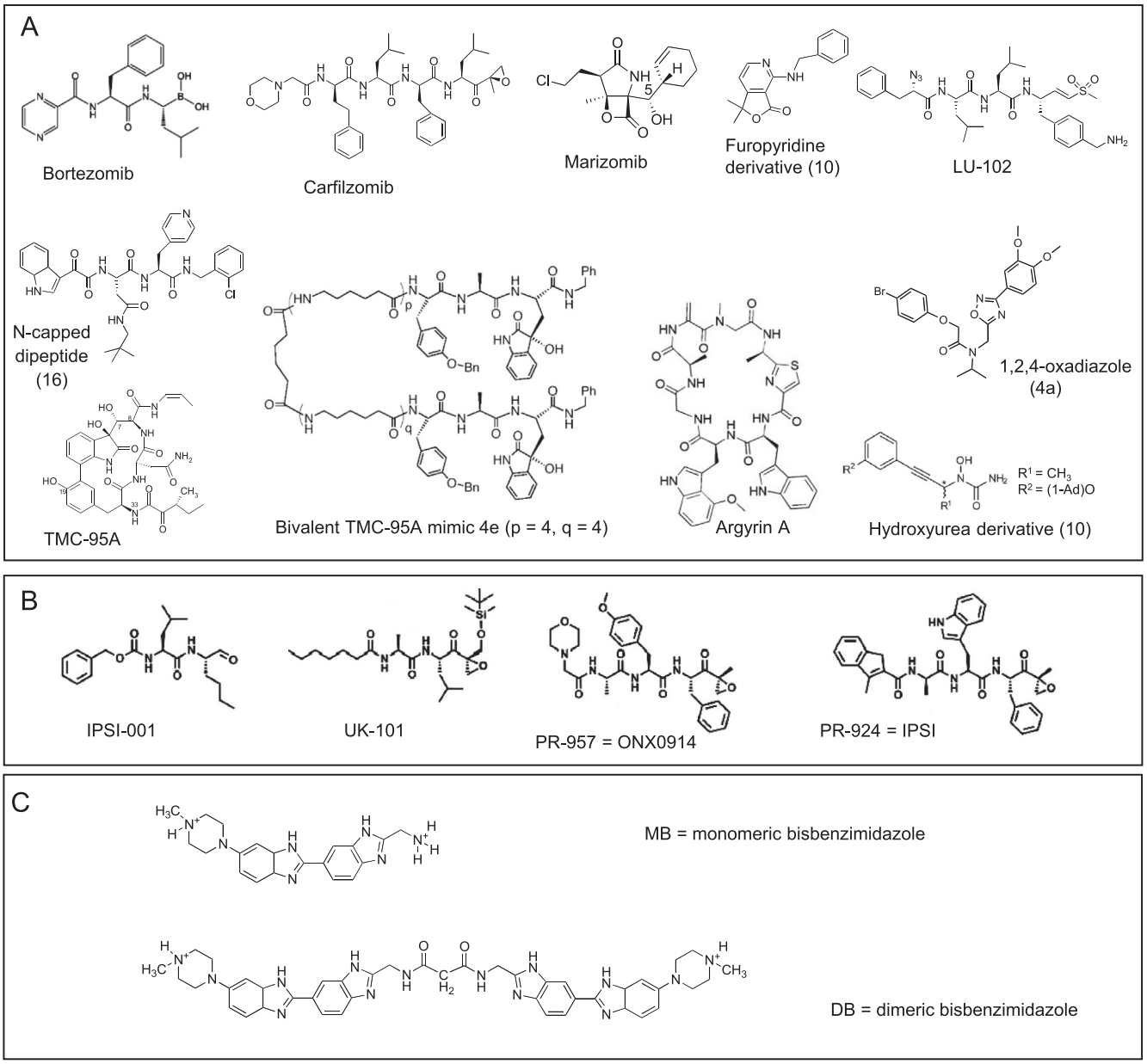

Fig. 1. Structures of established inhibitors of the constitutive proteasome (A), of the immunoproteasome (B) and of the two bisbenzimidazoles investigated in this study (C).

working for the recently described furopyridine γ-lactone 10 [23] (Fig. 1A) and for the peptide vinyl-sulfone LU-102 [24] (Fig. 1A) which are respectively efficient inhibitors of the PA and T-L activities of the c20S. In order to avoid possible cytotoxic effects on normal cells due to stable complex formation with these covalent inhibitors, non-covalent inhibitors [25–31] (Fig. 1A, lower part) and site-specific inhibitors of the c20S proteasome have been developed [3,6,23,32,33]. Some examples of promising non-covalent inhibitors are the N-capped dipeptide [28], the cyclic tripeptide TMC-95A [34] and its linearized and dimerized TMC-95A mimics (**4e**) [30], the cyclical peptide Argyrin A [35], the 1,2,4-oxadiazoles such as **4a** [26], and a hydroxyurea derivative (**10**) [25] (Fig. 1A).

Furthermore, new molecules are currently being developed searching to selectively target the immunoproteasome core particle (i20S) which is transiently expressed in cells under cytokine induction or permanently expressed in immunity-related cells [36,37]. The two proteolytic cores (c20S and i20S) are similarly structured [38]: the β1c, β2c and β5c being replaced by the β1i (LMP2), β2i (MECL-1) and β5i (LMP7) subunits. A number of proteasome inhibitors (PI) cannot discriminate between the constitutive proteasome and its immunoisoform. This is the case for bortezomib and carfilzomib, causing serious toxicity problems due to the major roles of constitutive proteasome in many cellular functions of normal cells. The smaller the doses used to induce apoptosis of the cancer cells, the less toxic the therapeutic treatment will be on the surrounding noncancerous cells. The only known selective inhibitors of the i20S catalytic sites are IPSI 001 (calpeptin) [39] (selective for β1i and β5i sites), UK101 specific for β1i sites [40], the epoxyketones ONX 0914 [41] and PR-924 [42] specific for β5i (Fig. 1B). No selective inhibitors of the β2i subsite have been found to date. Thus, there is a big challenge to find selective, site-specific inhibitors of this β2i i20S site and more generally to find specific inhibitors of immunoproteasome core particle.